Journal of Medical Virology 53:1–3 (1997)

HSV-1 and HSV-2 in Herpes Simplex Encephalitis: A Study of Sixty-Four Cases in the United Kingdom

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The incidence of herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2) in herpes simplex encephalitis (HSE) was investigated using cerebrospinal fluid (CSF) samples from sixty-four cases of HSE. A polymerase chain reaction (PCR) employing primers flanking a region of the HSV thymidine kinase gene common to both HSV-1 and HSV-2 was used to detect HSV in the CSF. HSV-1 and HSV-2 were differentiated by digestion with restriction enzymes. Two enzymes were employed; AvaI which cleaved only the HSV-2 gene product and AvaII which cleaved only the HSV-1 gene product. Sixty-three cases of HSE were found to be due to HSV-1; one case due to HSV-2. These data confirm previous observations that HSV-2 is a rare cause of post-neonatal herpes encephalitis but indicates that a PCR procedure capable of detection of both viruses is essential for efficient diagnosis of HSE. ***J. Med. Virol. 53:1–3, 1997.***

KEY WORDS: herpes simplex encephalitis; HSV-1; HSV-2; polymerase chain reaction; restriction endonuclease analysis; virus typing

INTRODUCTION

The majority of cases of herpes simplex encephalitis (HSE) occurring outside the neonatal period are thought to be due to herpes simplex type 1 (HSV-1) [Wolontis et al., 1977]. However, herpes simplex type 2 (HSV-2) can also be a cause of HSE. In a study of 103 brain biopsy confirmed cases of non-neonatal HSE, Nahmias et al. [1990] found 3.9% (four cases) were due to HSV-2. Since the introduction of the antiviral drug acyclovir [Skoldenberg et al., 1984; Whitley et al., 1986] for the treatment of herpes encephalitis the number of cases diagnosed by brain biopsy (and also at autopsy) has progressively declined. In consequence, the opportunity to determine, via culture, the type of virus causing HSE has also been reduced. Alternative non-invasive methods of diagnosis of HSE, such as determining specific intrathecal synthesis of HSV antibody [Klapper et al., 1981; Skoldenberg et al., 1981], do not ordinarily allow the differentiation of HSV-1 and HSV-2 caused HSE.

Early, specific, rapid diagnosis of HSE may now be achieved by polymerase chain reaction (PCR)-mediated detection of herpes simplex virus DNA in CSF [Puchhammer-Stöckl, 1995]. Using primers flanking a region of the HSV thymidine kinase gene common to HSV-1 and HSV-2 [Klapper et al., 1990], PCR was used to detect HSV DNA in CSF from 64 cases of HSE occurring in the United Kingdom (UK). Restriction enzyme (RE) digestion of the amplicons was used to differentiate HSV-1 or HSV-2.

MATERIALS AND METHODS

CSF Samples

CSF samples originally obtained from 64 patients with proven HSE diagnosed by brain biopsy, or by detection of intrathecally-synthesized HSV-specific antibody [Klapper et al., 1981] collected over a ten year period from centers throughout the UK were used in this study. CSF specimens were stored at −40°C and only those collected less than 12 days after onset of neurological illness were used. One case of HSV-2 meningitis was used as a positive control for HSV-2 genotyping.

The age of patients in the study ranged from 1 to 75 years; mean: 39.87 years. The group comprised of 31 males and 33 females.

PCR

DNA was purified from CSF by phenol/chloroform extraction and ethanol precipitation [Dennett et al., 1991] and PCR was performed as previously described [Klapper et al., 1990]. A 351 base pair fragment was amplified following 50 cycles of 94°C/2 min; 50°C/1.5 min; and 70°C/2 min.

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Accepted 6 March 1997

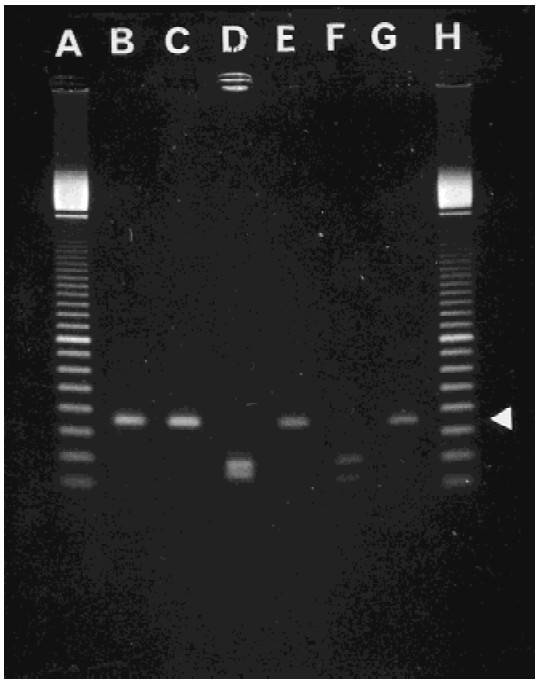

Fig. 1. Agarose gel stained with 1 mg/ml ethidium bromide. Arrowhead denotes 351 bp. **Lanes A** and **H**: 100 base pair DNA ladder. **Lane B**: HSV-1 amplicon. **Lane C**: HSV-1 amplicon + AvaI. **Lane D**: HSV-1 amplicon + AvaII. **Lane E**: HSV-2 amplicon. **Lane F**: HSV-2 amplicon + AvaI. **Lane G**: HSV-2 amplicon + AvaII.

Restriction Endonuclease Typing

20 μl of PCR product was digested with two units of either restriction enzyme Ava I or Ava II (Boehringer Mannheim, East Sussex, UK) at 37°C for two hrs in a buffer comprising: 10 mM Tris-HCl pH 7.5; 10 mM $MgCl_2$; 50 mM NaCl; and 1 mM dithioerythritol. Resulting fragments were separated by agarose gel electrophoresis (1.5% agarose in Tris-acetate-EDTA buffer run at 5 V/cm for 1 hr). After staining with ethidium bromide the gel was visualised by transillumination.

RESULTS AND DISCUSSION

AvaII will only cut an HSV-1 amplicon and AvaI cuts an HSV-2 amplicon (Fig. 1). All 64 samples gave positive PCR results. Sixty-three products were cleaved with restriction enzyme Ava II; one was cleaved by Ava I. This indicates that, in this study, only 1 case of HSE in 64 was due to HSV-2.

The results of this study suggest that in the UK approximately 2% of cases (1 of 64) of HSE are caused by HSV-2. This compares with an incidence of 3.9% (4 of 103 cases) reported for the United States in 1982 in a study utilizing virus isolated from brain biopsy material [Nahmias et al., 1990], and an incidence of 6.5% (6 of 93 cases) reported in a study conducted in Sweden [Aurelius et al., 1993]. The former study typed virus isolated by brain biopsy while the latter study used a PCR detection of HSV DNA in CSF.

The relative proportions of HSV-1 and HSV-2 HSE found in this and in the studies described above may reflect the generally lower incidence of primary HSV-2 infections relative to primary HSV-1 infection in the community. Serological studies from the United States suggest a 20% seroprevalence of HSV-2 antibodies [Johnson et al., 1989] in comparison with rates of 90% or more for HSV-1 antibodies [Nahmias et al., 1990]. British studies reported that 22.7% of genito-urinary clinic attenders and 7.6% blood donors had antibodies to HSV-2 [Cowan et al., 1994]. Using samples from a variety of sources, overall rates of 80% for HSV-1 antibodies and 10.4% for HSV-2 antibodies were found by Ades et al. [1989]. The 4:1 incidence shown by the studies of Johnson et al. [1989] and Nahmias et al. [1990] might suggest that 20% of cases of HSE could be due to HSV-2. However, in this and other studies lower rates of HSV-2 caused HSE have been found. The relative frequency of HSV-1 and HSV-2 in HSE suggest that HSV-1 and HSV-2 may exhibit different patterns of neuropathogenesis.

A variety of methods can be used for typing of PCR amplified HSV DNA. Aurelius et al. [1993] employed two PCR's utilizing oligonucleotides specific for HSV-1 or for HSV-2. Other approaches include amplification of a segment of DNA common to the human herpesviridae (HSV-1, HSV-2, VZV, EBV, CMV, HHV-6) and HSV differentiated by restriction enzyme digestion [Rozenberg and Lebon, 1992] or nested multiplex PCR [Tenorio et al., 1993]; hybridization of PCR amplicons with type-specific probes [Shoji et al., 1994]; or simultaneous amplification of DNA from both HSV-1 and HSV-2 using the upstream primer common to both and downstream primers specific for either one or the other [Shimizu et al., 1994] with different size amplicons being produced from HSV-1 and HSV-2 and the identity confirmed by RE digestion. In our study, two restriction enzymes were used; AvaI which will cleave only the HSV-2 amplicon and AvaII which cleaves only the HSV-1 amplicon. Two restriction enzymes were used firstly because Taq DNA polymerase does not exhibit proof reading activity during copying, thus introducing the possibility of transcription errors during amplification [Krawczak et al., 1989], the risk increasing when the number of target molecules is low (as in the detection of HSV DNA in CSF) and secondly, because a point mutation within the original target DNA could render the amplicon falsely resistant to RE digestion.

The non-invasive nature of PCR diagnosis may allow the identification of previously unrecognized forms of HSV infection [Young et al., 1992] and the differentiation of the two types of HSV involved in such disease may be useful to adequately study these cases. Typing of HSV has been shown to be particularly important in the study of neonatal HSE [Corey et al., 1988; Malm et al., 1991]. In a study of 24 infants treated for neonatal HSE, the proportion reported to have HSV-2 encephalitis (62.5%) presented with more severe symptoms than those with HSV-1 encephalitis and only 23% of those with HSV-2 were normal at follow-up compared with 100% HSV-1 patients [Corey et al., 1988].

Even though HSV-2 appears to be responsible for

only 2–4% of cases of HSE in adults, it is apparent that if potentially treatable cases of HSE are not to be missed, PCR methods for diagnosis of HSE must be capable of detection of both HSV-1 and HSV-2.

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